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Liposome-Mediated Introduction of Foreign Proteins into the Matrix of Protoplasts and into the Peroxisomal Membrane of the Methanol-Grown Yeast *Hansenula polymorpha*

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is made of clinical quality PMMA and is sutured episclerally with eight stainless steel wires. Since PMMA is hydrophobic, easily damaged and difficult to sterilize, we designed, developed and tested a new KP made of glass and fitted in a metal cylinder with flange of either Inconel-600 or technically pure platinum. The anterior curvature of the glass was shaped by pressure-moulding or melting.

Results: The optics of the melted glass core are superior to the moulded one and, moreover, contact between glass and metal was better. The Inconel-600 KP caused melting away and ulceration of the surrounding cornea, most likely due to metal ions. With the platinum-glass KP the only complication, if present, was corneal overgrowth.

Conclusion: SEM images show that over a period of at least five months the platinum-glass KP did not show leakage, extrusion or adverse tissue reactions such as apocification, melting away, infection or epithelial downgrowth. From these data it would seem that a clinical trial of the presently available newly developed platinum-glass KP should be undertaken.

MORPHOLOGY OF LINEAR-LOW-DENSITY POLYETHYLENE (LLDPE)

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The distribution of comonomer units in the chains of LLDPE is known to influence the crystallization and melting behaviour. Typical Differential Scanning Calorimetry (DSC) curves feature a broad melting temperature range and, moreover, show two major peaks. It has been shown that the crystallite size distribution suggested by these curves is caused by intermolecular heterogeneity of the comonomer distribution. This intermolecular heterogeneity should thus be reflected in the lamellar morphology of a LLDPE sample.

Samples of Stamylex 1016 with several known thermal histories were fixed and stained by means of the usual HClSO_3 treatment in the vapour phase. Sections of about 60 nm were obtained at room temperature and stained with Uranyl acetate.

Observation in CTEM clearly shows

that the cooling rate affects the lamellar thickness and sizes: a distinct lamellar thickening is observed in samples which have been isothermally crystallized below the highest melting peak. It is at this temperature that the chains with low comonomer content crystallize. These results were confirmed by means of a different fixation/staining method in bulk using RuO_4 .

LIPOSOME-MEDIATED INTRODUCTION OF FOREIGN PROTEINS INTO THE MATRIX OF PROTOPLASTS AND INTO THE PEROXISOMAL MEMBRANE OF THE METHANOL-GROWN YEAST HANSENULA POLYMORPHA

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Low-pH-induced fusion between liposomes and protoplasts or isolated peroxisomes of the methanol-grown yeast Hansenula polymorpha could be demonstrated using a fluorescent assay and freeze-etch techniques. Best results were obtained with liposomes composed of cardiolipin and phosphatidylcholine.

This method was used for the introduction of foreign proteins into protoplasts and peroxisomes. After fusion or glucose oxidase containing liposomes with protoplasts, glucose oxidase activity was demonstrated in the cytosol of the protoplasts with cytochemical techniques. Similarly ferritin could be introduced into the cytosol of protoplasts and also into the matrix of isolated peroxisomes. If ferritin was added to protoplasts together with empty liposomes, this protein was also introduced into the protoplasts. However, in the absence of liposomes no uptake of ferritin occurred.

In addition, it was demonstrated that liposomes can be used as a carrier to introduce foreign proteins into the membrane of isolated peroxisomes. After fusion of bacteriorhodopsin-containing liposomes with isolated peroxisomes freeze-etch experiments revealed the presence of large protein particles in the peroxisomal membrane which normally shows largely smooth fracture faces.

These techniques are currently being used in the study of the biogenesis of yeast peroxisomes.